



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Applicant : Kathryn K. Lappegard
Filed : 11/22/2000
TC/A.U. : 1638
Examiner : Baum, Stuart F.
Docket No. : 1189EE
Customer No. : 27310
Title : Seed-Preferred Regulatory Elements and Uses Thereof

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF SHANE E. ABBITT UNDER 37 CFR § 1.132

I, Shane E. Abbitt, declare:

I am a citizen of the United States of America, and a resident of Ankeny, Iowa, United States of America.

I received the degree of Master of Science in the area of Life Sciences (Biotechnology) from the University of Tennessee, Knoxville, Tennessee, 1996.

I received the degree of Bachelor of Arts from Simpson College, Indianola, Iowa, 1993.

I presently hold the position of Senior Research Associate at Pioneer Hi-Bred International, Inc. May 1999 - present. Research areas: 1) Seed specific promoter isolation and 2) Vector construction.

I was employed as Research Associate at Pioneer Hi-Bred International, Inc. September 1997 – May 1999. Research areas: Vector construction.

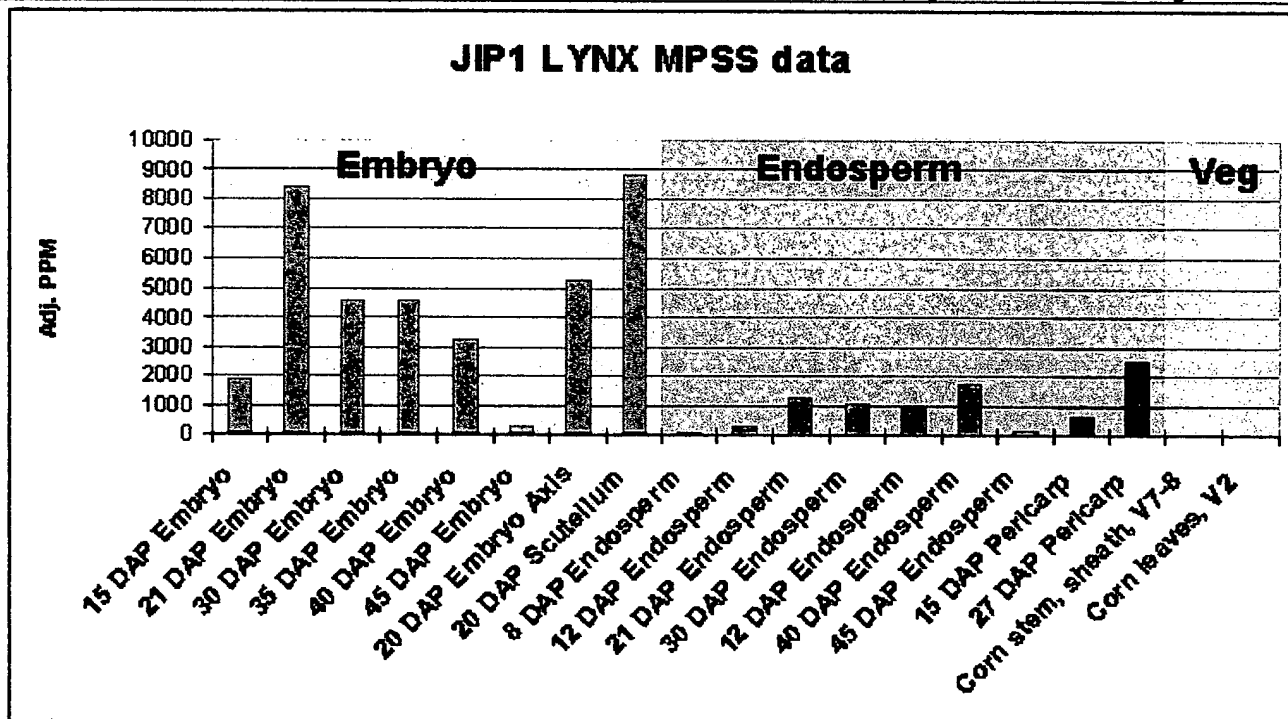
I was employed as a **Temporary Lab Assistant** at Pioneer Hi-Bred International, Inc. February 1997 – September 1997. Research areas: Hybridization and expression analysis.

I was employed as a **Research Assistant** at the University of Tennessee February 1996 – January 1997. Research areas: DNA fingerprinting of floriculture species.

I am personally familiar with the method of collection of the data provided below:

Fig 1:

MPSS data indicates that the JIP1 transcript is present from 15 – 40 DAP (days after pollination) in the embryo and aleurone. The signal from endosperm and pericarp is that of the aleurone. No signal is detected in vegetative tissues.



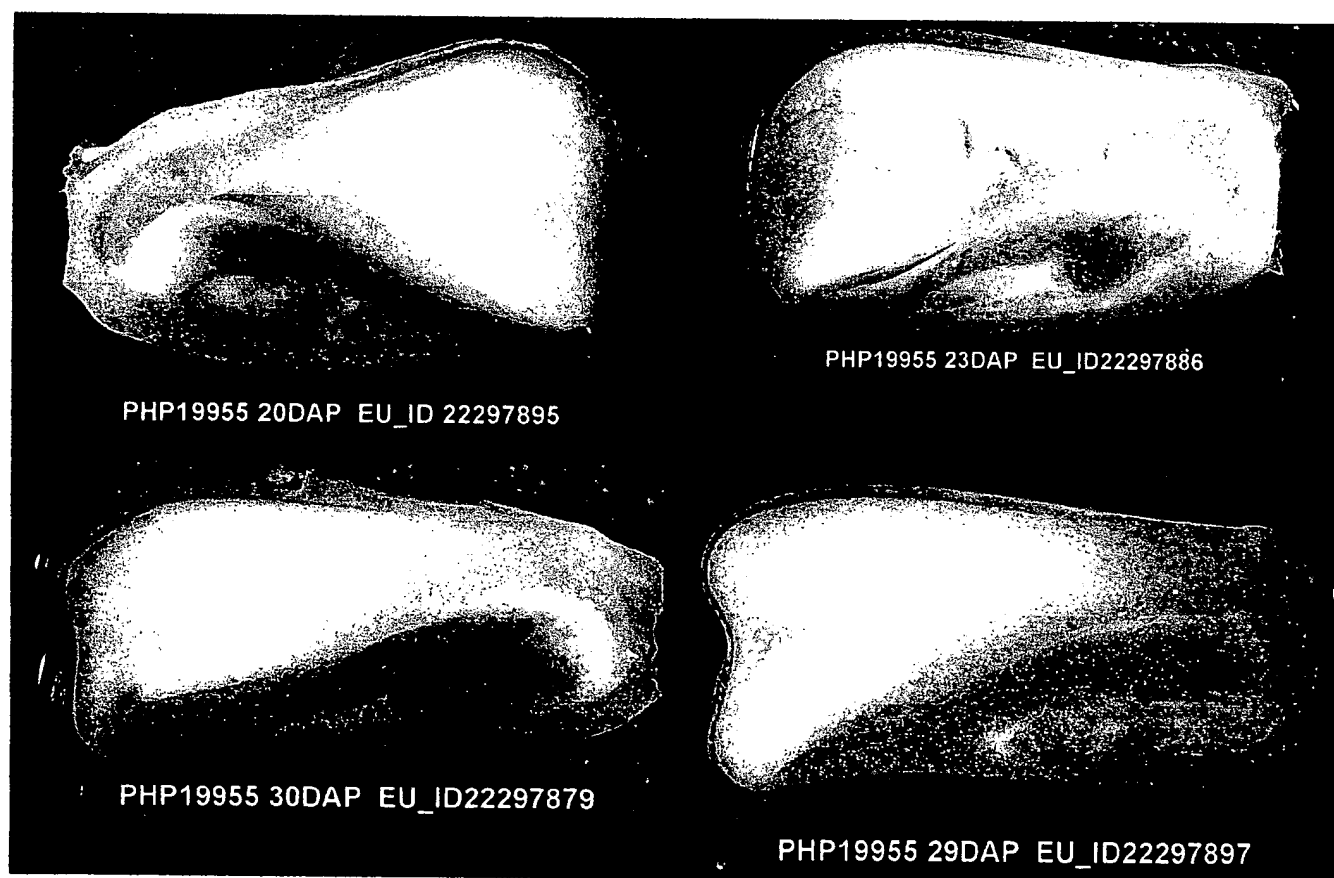
The JIP1 regulatory regions were isolated based on MPSS (Massively Parallel Signature Sequencing) technology from LYNX™ (see Brenner *et al*, Nature Biotechnology 18:630-634, 2000) expression analysis showing JIP1 was predominantly

expressed in the embryo and aleurone from 15 – 40 DAP. The top BLAST hit to the JIP1 open reading frame is a Jasmonate Induced Protein.

The JIP1B promoter (SEQ ID NO:1) was isolated from maize using the Genome Walker protocol as described in Example 1 of the specification (page 19, beginning on line 23).

The expression pattern predicted by the MPSS analysis and Northern blot analysis (described in Example 6 of the specification, page 31, line 24) was confirmed in stable transgenic experiments where this promoter was fused to GUSINT as described in Example 3 of the specification (page 22, line 1). No signal was detected in vegetative tissue: tassel, leaf, and primary root.

Fig 2: Transgenic Expression: the JIP1B promoter (SEQ ID NO:1) fused to GUSINT::pinII

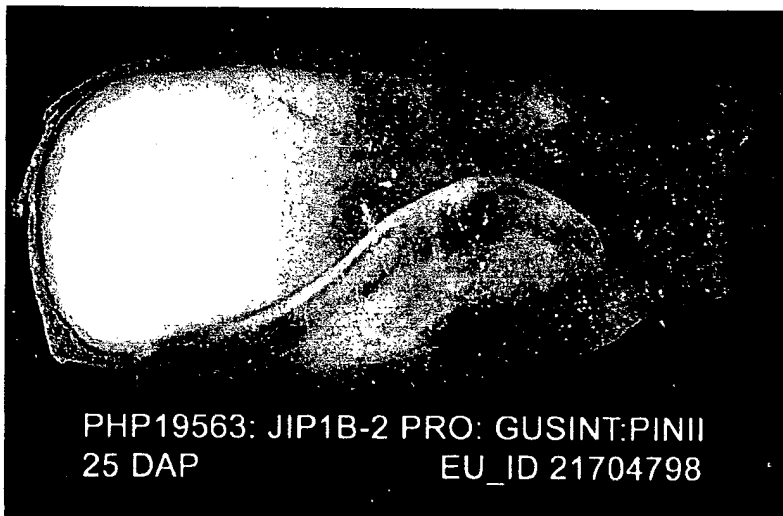


The JIP1B-2 promoter was made by truncating the JIP1B promoter (SEQ ID NO:1) at the XbaI site (TCTAGA) at position 514, creating a fragment including positions 514-1247 of SEQ ID NO:1.

For both promoter versions, A was changed to C at position 1247, to create an NcoI site.

This fragment was also fused to GUSINT as described in Example 3 of the specification. Transgenic expression at 25 DAP (days after pollination) was seen in the embryo and aleurone tissues, confirming that this was a functional fragment of the promoter as disclosed in SEQ ID NO:1. No signal was detected in leaf vegetative tissue.

Fig. 3: Transgenic expression of Jip1B (truncated)::GUSINT::pinII:



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Group Art Unit: 1638

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.



Shane E. Abbitt

Dated: 10/31/05